# RNA isolation

## User manual

NucleoSpin® RNA XS

September 2015 / Rev. 09

MACHEREY-NAGEL
www.mn-net.com

# RNA isolation

## Protocol-at-a-glance (Rev. 09)

| | | | | XS |
|---|---|---|---|---|
| | | | | **NucleoSpin® RNA XS** |
| **1** | **Supply sample** | | | Use up to $10^5$ cultured cells or 5 mg tissue samples |
| **2** | **Lyse and homogenize cells** | | | 100 µL RA1<br>2 µL TCEP<br>Mix |
| **3** | **Add Carrier RNA** | | | 5 µL Carrier RNA working solution<br>Mix |
| **4** | **Filtrate lysate** *(optional)* | |  | 11,000 x *g*, 30 s |
| **5** | **Adjust RNA binding condition** | | | 100 µL 70 % ethanol<br>Mix |
| **6** | **Bind RNA** | |  | Load lysate<br>11,000 x *g*, 30 s |
| **7** | **Desalt silica membrane** | |  | 100 µL MDB<br>11,000 x *g*, 30 s |
| **8** | **Digest DNA** | | | 25 µL DNase reaction mixture<br>RT, 15 min |
| **9** | **Wash and dry silica membrane** | |  | 1st wash: 100 µL RA2, RT, 2 min, 11,000 x *g*, 30 s<br>2nd wash: 400 µL RA3, 11,000 x *g*, 30 s<br>3rd wash: 200 µL RA3, 11,000 x *g*, 2 min |
| **10** | **Elute highly pure RNA** | |  | 10 µL RNase-free $H_2O$<br>11,000 x *g*, 30 s |

**MACHEREY-NAGEL GmbH & Co. KG** · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany
Tel.: +49 24 21 969-270 · Fax: +49 24 21 969-199 · tech-bio@mn-net.com · ***www.mn-net.com***

# Table of contents

1 Components 4
1.1 Kit contents 4
1.2 Reagents, consumables, and equipment to be supplied by user 5
1.3 About this user manual 5
2 Product description 6
2.1 The basic principle 6
2.2 Kit specifications 6
2.3 Handling, preparation, and storage of starting materials 9
2.4 Elution procedures 10
2.5 Stability of isolated RNA 10
3 Storage conditions and preparation of working solutions 11
4 Safety instructions 13
5 Protocols 16
5.1 RNA purification from cultured cells, laser captured cells, or microdissected cryosections 16
5.2 RNA purification from tissue 19
5.3 Clean-up and concentration of RNA 23
5.4 rDNase digestion in the eluate 25
6 Appendix 27
6.1 Troubleshooting 27
6.2 Ordering information 31
6.3 References 32
6.4 Product use restriction / warranty 32

# 1 Components

## 1.1 Kit contents

| | NucleoSpin® RNA XS | | |
|---|---|---|---|
| **REF** | **10 preps 740902.10** | **50 preps 740902.50** | **250 preps 740902.250** |
| Lysis Buffer RA1 | 6 mL | 25 mL | 125 mL |
| Wash Buffer RA2 | 2 x 1 mL | 15 mL | 2 x 15 mL |
| Wash Buffer RA3 (Concentrate)* | 6 mL | 12 mL | 50 mL |
| Membrane Desalting Buffer MDB | 10 mL | 10 mL | 50 mL |
| Reaction Buffer for rDNase | 7 mL | 7 mL | 30 mL |
| rDNase, RNase-free (lyphilized)* | 1 vial (size A) | 1 vial (size C) | 2 vials (size D) |
| Carrier RNA* | 300 µg | 300 µg | 300 µg |
| Reducing Agent TCEP* | 14 mg | 3 x 14 mg | 2 x 107 mg |
| RNase-free $H_2O$ | 13 mL | 13 mL | 13 mL |
| NucleoSpin® Filters (violet rings) | 10 | 50 | 250 |
| NucleoSpin® RNA XS Columns (light blue rings – plus Collection Tubes) | 10 | 50 | 250 |
| Collection Tubes (2 mL) | 30 | 150 | 750 |
| Collection Tubes (1.5 mL) | 10 | 50 | 250 |
| User manual | 1 | 1 | 1 |

* For preparation of working solutions and storage conditions see section 3.

## 1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96–100 % ethanol (to prepare Wash Buffer RA3 and for the clean-up procedure, section 5.3)
- 70 % ethanol (to adjust RNA binding condition)

Consumables

- 1.5 mL microcentrifuge tubes
- Sterile RNase-free tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Personal protection equipment (lab coat, gloves, goggles)

## 1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® RNA XS** kit is used for the first time. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

# 2 Product description

## 2.1 The basic principle

One of the most important aspects isolating RNA is to prevent degradation of the RNA during the isolation procedure. With the **NucleoSpin® RNA** methods, cells are lysed by incubation in a solution containing large amounts of chaotropic ions. This lysis buffer immediately inactivates RNases – which are present in virtually all biological materials – and creates appropriate binding conditions which favor adsorption of RNA to the silica membrane. Contaminating DNA, which is also bound to the silica membrane, is removed by an rDNase solution which is directly applied onto the silica membrane during the preparation (RNase-free rDNase is supplied with the kit). Simple washing steps with two different buffers remove salts, metabolites and macromolecular cellular components. Pure RNA is finally eluted under low ionic strength conditions with RNase-free $H_2O$ (supplied).

The RNA preparation using **NucleoSpin® RNA** kits can be performed at room temperature. The eluate, however, should be treated with care because RNA is very sensitive to trace contaminations of RNases, often found on general lab ware, fingerprints and dust. To ensure RNA stability, keep RNA frozen at -20 °C for short-term or -70 °C for long-term storage.

## 2.2 Kit specifications

- The **NucleoSpin® RNA XS** kit is recommended for the isolation of RNA from very small samples. Typical sample material comprises small amounts of cells (up to $1 \times 10^5$) and tissue (up to 5 mg) such as pellets of cultured cells, laser-captured cells, microdissected cryosections, biopsy samples, fine needle aspirates, and flow cytometer sorted cells (Table 1, page 7).
- The innovative column design with a funnel shaped thrust ring and a small silica membrane area allows elution of RNA in as little as 5–30 µL. Thus, **highly concentrated RNA** is eluted, ready for common downstream applications (e.g., RT-PCR).
- The **RNA yield** strongly depends on the sample type, quality, and amount (see Table 2, page 8 for details).
- **High quality RNA** (RNA Integrity Number (RIN) > 9 according to Agilent 2100 Bioanalyzer assays) can be obtained from small samples (e.g., $10^3$ cells, 0.1 mg tissue) as well as from larger samples ($10^5$ cells, 5 mg tissue). rRNA ratios (28S / 18S) of 1.8–2.0 can be obtained. Since RNA quality always depends on the sample quality, see section 6.3 for further aspects.
- The **NucleoSpin® RNA XS** kit allows purification of RNA with an $A_{260}/A_{280}$ ratio generally exceeding 1.9 (measured in TE buffer pH 7.5). Due to the high RNA **purity** large amounts of eluates can be used as template in RT-PCR without inhibition (e.g., 8 µL of 10 µL eluates as template in a 20 µL qRT-PCR setup generating stronger signal compared to reactions with less template in a LightCycler PCR with the Sigma SYBR Green Quantitative RT-PCR Kit).

- The **preparation time** is approximately 45 min for 12 samples.
- As **Reducing Agent TCEP** (Tris(2-carboxyethyl)phosphine) is supplied in the kit. TCEP is odorless, more stable, more specific for disulfide-bonds, and less toxic than other commonly used reducing agents.
- **Carrier RNA** (poly(-A) RNA: poly(A) potassium salt, prepared from ADP with polynucleotide phosphorylase) is included for optimal performance with smallest samples.

  It is recommended adding Carrier RNA to the sample lysate (20 ng per sample). Such small amounts typically do not interfere with subsequent RT-PCR, even in oligo-dT primed reverse transcriptions. The small amount of Carrier RNA transfered into a reverse transcription reaction is commonly not significantly influencing the outcome of the reaction, due to the large excess of oligo-dT primer. The benefit of adding Carrier RNA to the sample lysate depends on sample type, amount, and kind of downstream RNA analysis. Carrier RNA should be omitted when subsequent to RNA isolation
  - a poly-A RNA isolation is performed
  - RNA sequencing is performed.
- **rDNase** is supplied in the kit. DNA contaminations are removed by on-column digestion with rDNase. For most demanding applications (e.g., expression analysis of plasmid transfected cells, plastidial or mitochondrial genes) a subsequent digestion with rDNase in the eluate is possible.

**Table 1: Kit specifications at a glance**

| Parameter | NucleoSpin® RNA XS |
|---|---|
| Format | Mini spin column – XS design |
| Sample material | Small amounts of tissue<br>< 5 mg tissue,<br>< 100 000 cultured cells |
| Fragment size | > 200 nt |
| Typical yield | See table 2 for examples |
| $A_{260}/A_{280}$ | 1.9–2.1 |
| Typical RIN<br>(RNA integrity number) | > 9 (depending on sample quality) |
| Elution volume | 5–30 µL |
| Preparation time | 35 min/6 preps |
| Binding capacity | 110 µg |

**Table 2: Overview on average yields of RNA isolation using NucleoSpin® RNA XS**

| Sample | Average yield |
|---|---|
| $10^5$ HeLa cells | 1000–1500 ng |
| $10^4$ HeLa cells | 100–150 ng |
| $10^3$ HeLa cells | 10–15 ng |
| $10^2$ HeLa cells | 0.1–1.5 ng |
| 5 mg mouse kidney | 5–8 µg |
| 1 mg mouse kidney | 2 µg |

## 2.3 Handling, preparation, and storage of starting materials

RNA is not protected against digestion until the sample material is flash frozen or disrupted in the presence of RNase inhibiting or denaturing agents. Therefore it is important that samples are flash frozen in liquid $N_2$ immediately and stored at -70 °C, or processed as soon as possible. Samples can be stored in Lysis Buffer RA1* (+ TCEP) after disruption at -70 °C for up to one year, at 4 °C for up to 24 hours or up to several hours at room temperature. Frozen samples are stable up to 6 months. Frozen samples in Buffer RA1* (+ TCEP) should be thawed slowly before starting with the isolation of RNA.

*Wear gloves at all times during the preparation. Change gloves frequently.*

**Cultured animal cells** are collected by centrifugation and directly lysed by adding Buffer RA1 according to step 2 of the standard protocol (see section 5).

**Cell lysis of adherent growing cells in a culture dish**

Completely aspirate cell-culture medium, and continue immediately with the addition of Lysis Buffer RA1 to the cell-culture dish. Avoid incomplete removal of the cell-culture medium in order to allow full lysis activity of the lysis buffer.

**To trypsinize adherent growing cells**

Aspirate cell-culture medium, and add an equal amount of PBS in order to wash the cells. Aspirate PBS. Add 0.1–0.3 % trypsin in PBS and incubate for an appropriate time to detach the cells from the dish surface. After cell detachment, add medium, transfer cells to an appropriate tube (not supplied), and pellet by centrifugation for 5 min at 300 x *g*. Remove supernatant and continue with the addition of lysis buffer to the cell pellet.

**Animal tissues** are often tough and should be disrupted mechanically to be available for lysis. Depending on the disruption method, the viscosity of the lysed sample has to be reduced further for optimal results. It is essential for efficient RNA preparation that all the RNA contained in the sample is released from the cells by disruption and that the viscosity of the sample is reduced by homogenization.

Thawing of undisrupted animal tissue should only be done in the presence of Buffer RA1 under simultaneous mechanical disruption, for example with a rotor-stator homogenizer or a bead mill. This ensures that the RNA is not degraded by RNases before the preparation has started.

Commonly used techniques for disruption of animal tissues are, for example grinding with **pestle and mortar** or using **a syringe and needle** for multiple passage of the sample through the needle. However, due to the small size of samples to be processed with **NucleoSpin® RNA XS** these disruption methods are often not suitable.

* Add TCEP optional before or after freezing.

**Recommended disruption and homogenization methods**

The simple addition of lysis buffer and subsequent vortexing is usually sufficient to disrupt and homogenize for example up to $10^4$ cultured cells, laser captured cells, or microdissected cryosections.

Tissue can be homogenized using a **rotor-stator homogenizer**. The spinning rotor disrupts and simultaneously homogenizes the sample which is submerged in lysis buffer by shearing within seconds up to minutes (homogenization time depends on sample). Keep the rotor tip submerged to avoid excess foaming. Select a suitably sized homogenizer (5–7 mm diameter rotors can be used for homogenization in microcentrifuge tubes).

**Bead-milling** disrupts the tissue samples, submerged in lysis buffer, by rapid agitation in the presence of beads. Suitable disruption parameters (type, size and number of beads, tube type, speed and time of agitation) have to be determined empirically for each application.

## 2.4 Elution procedures

A high RNA concentration in the elution fraction is desirable for all typical downstream applications. In particular with regard to limited volumes of reaction mixtures, high RNA concentration can be a crucial criterion. Due to a high default elution volume, standard kits often result in weakly concentrated RNA, if only small samples are processed.

Such RNA often even requires a subsequent concentration to be suitable for the desired application.

In contrast to standard kits, **NucleoSpin® RNA XS** allows an efficient elution in a very small volume resulting in highly concentrated RNA.

Elution volumes in the range of 5–30 µL are recommended, the default volume is 10 µL.

## 2.5 Stability of isolated RNA

Eluted RNA should immediately be put and always kept on ice during work for optimal stability! Contamination with almost omnipresent RNases (general lab ware, fingerprints, dust) may be a risk for isolated RNA. For short-term storage freeze at -20 °C, for long-term storage freeze at -70 °C.

# 3 Storage conditions and preparation of working solutions

**Attention:** *Buffers RA1, RA2, and MDB contain chaotropic salt and detergents. Wear gloves and goggles!*

*CAUTION: Buffers RA1, RA2, and MDB contain guanidinium thiocyanate which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.*

- Store lyophilized **rDNase, Reducing Agent TCEP**, and **Carrier RNA** at 4 °C on arrival (stable up to 1 year).
- All other kit components should be stored at room temperature (18–25 °C) and are stable up to one year. Storage at lower temperatures may cause precipitation of salts.
- Check that 70 % ethanol is available as additional solution in the lab to adjust RNA binding conditions in the Buffer RA1 lysate.
- Check that 96–100 % ethanol is available (necessary for clean-up protocol only).

Before starting with any **NucleoSpin® RNA XS** protocol prepare the following:

- **rDNase:** Add indicated volume (see following table or label on the rDNase vial) of RNase-free $H_2O$ to the rDNase vial and incubate for 1 min at room temperature. Gently swirl the vials to completely dissolve the rDNase. Be careful not to mix rDNase vigorously as rDNase is sensitive to mechanical agitation. Dispense into aliquots and store at -20 °C. The frozen working solution is stable for 6 months. Do not freeze/thaw the aliquots more than three times.
- **Reducing Agent TCEP:** Add indicated volume of RNase-free $H_2O$ to the TCEP vial and incubate for several minutes at room temperature. Mix the vial to completely dissolve the TCEP. Store dissolved TCEP at -20 °C.
- **Carrier RNA:** Prepare a **stock solution** before first time using: Dissolve the Carrier RNA* in 750 µL Buffer RA1 to obtain a 400 ng/µL stock solution. Prepare a **working solution** before RNA extraction: Dilute 1:100 with Buffer RA1 (e.g., 1 µL Carrier RNA stock solution + 99 µL Buffer RA1) to obtain the working solution of 4 ng/µL. Add 5 µL of this working solution (20 ng) to every lysate (protocol step 3 in section 5). Store stock solution at -20 °C; do not store working solution, prepare it freshly immediately before use.
- **Wash Buffer RA3:** Add the indicated volume of 96–100 % ethanol to Wash Buffer RA3 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer RA3 at room temperature (18–25 °C) for up to one year.

* Due to the production procedure, lyophilized Carrier RNA might hardly be visible in the vial.

| NucleoSpin® RNA XS | | | |
|---|---|---|---|
| **REF** | **10 preps<br>740902.10** | **50 preps<br>740902.50** | **250 preps<br>740902.250** |
| Wash Buffer RA3 (Concentrate) | 6 mL<br>Add 24 mL ethanol | 12 mL<br>Add 48 mL ethanol | 50 mL<br>Add 200 mL ethanol to each bottle |
| rDNase, RNase-free (lyophilized) | 1 vial (size A)<br>Add 55 µL RNase-free $H_2O$ | 1 vial (size C)<br>Add 230 µL RNase-free $H_2O$ | 2 vials (size D)<br>Add 540 µL RNase-free $H_2O$ to each vial |
| Carrier RNA | 300 µg | 300 µg | 300 µg |
| | Add 750 µL Buffer RA1 to obtain concentrated stock solution. Dilute 1:100 with Buffer RA1 to obtain working solution. | | |
| Reducing Agent TCEP | 14 mg<br>Add 100 µL RNase-free $H_2O$ | 3 x 14 mg<br>Add 100 µL RNase-free $H_2O$ to each vial | 2 x 107 mg<br>Add 750 µL RNase-free $H_2O$ to each vial |

# 4 Safety instructions

The following components of the NucleoSpin® RNA XS kits contain hazardous contents.

*Wear gloves and goggles and follow the safety instructions given in this section.*

**GHS classification**

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.
*Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.*

| Component<br>*Inhalt* | Hazard contents<br>*Gefahrstoff* | GHS symbol<br>*GHS-Symbol* | Hazard phrases<br>*H-Sätze* | Precaution phrases<br>*P-Sätze* |
|---|---|---|---|---|
| MDB | Guanidinium thiocyanate + ethanol 5–20 %<br>*Guanidinthiocyanat 1–15 % + Ethanol 5–20 %*<br>CAS 593-84-0, 64-17-5d | <br>WARNING<br>*ACHTUNG* | 226 | 210, 233, 370+378, 403+235 |
| RA1 | Guanidinium thiocyanate 30–60 %<br>*Guanidinthiocyanat 30–60 %*<br>CAS 593-84-0 | <br>WARNING<br>*ACHTUNG* | 302, 412, EUH031 | 260, 273, 301+312, 330 |
| RA2 | Guanidinium thiocyanate 30–60 % + ethanol 20–35 %<br>*Guanidinhydrochlorid 24–36 % + Ethanol 20–35 %*<br>CAS 593-84-0, 64-17-5 | <br>WARNING<br>*ACHTUNG* | 226, 302, 412, EUH031 | 210, 233, 260, 273, 301+312, 330, 370+378, 403+235 |
| rDNase | rDNase 90–100 %<br>*rDNase 90–100 %*<br>CAS 9003-98-9 | <br>DANGER<br>*GEFAHR* | 317, 334 | 261, 280, 302+352, 304+340, 333+313, 342+311, 363 |
| TCEP | tris-(2-carboxyethyl) phosphine hydrochloride, TCEP(•HCl) 70–100 %<br>*Tris-(2-carboxyethyl) phosphinhydrochlorid, TCEP(•HCl) 70–100 %*<br>CAS 51805-45-9 | <br>WARNING<br>*ACHTUNG* | 315, 319 | 264, 280, 302+352, 305+351+338, 332+313, 337+313 |

**Hazard phrases**

H226 Flammable liquid and vapour.
*Flüssigkeit und Dampf entzündbar.*

H302 Harmful if swallowed.
*Gesundheitsschädlich bei Verschlucken.*

| | |
|---|---|
| H315 | Causes skin irritation.<br>*Verursacht Hautreizungen.* |
| H317 | May cause an allergic skin reaction.<br>*Kann allergische Hautreaktionen verursachen.* |
| H319 | Causes serious eye irritation.<br>*Verursacht schwere Augenreizung.* |
| H334 | May cause allergy or asthma symptoms or breathing difficulties if inhaled.<br>*Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.* |
| H412 | Harmful to aquatic life with long lasting effects.<br>*Schädlich für Wasserorganismen, mit langfristiger Wirkung.* |

**Precaution phrases**

| | |
|---|---|
| P 210 | Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.<br>*Von Hitze, heissen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.* |
| P233 | Keep container tightly closed.<br>*Behälter dicht verschlossen halten.* |
| P260 | Do not breathe dust/fume/gas/mist/vapours/spray.<br>*Staub/Rauch/Gas/Nebel/Dampf/Aerosol nicht einatmen.* |
| P261 | Avoid breathing dust/fume/gas/mist/vapours/spray.<br>*Einatmen von Staub/Rauch/Gas/Nebel/Dampf/Aerosol vermeiden.* |
| P264 | Wash … thoroughly after handling.<br>*Nach Handhabung … gründlich waschen.* |
| P273 | Avoid release to the environment.<br>*Freisetzung in die Umwelt vermeiden.* |
| P280 | Wear protective gloves/protective clothing/eye protection/face protection.<br>*Schutzhandschuhe/Schutzkleidung/Augenschutz/Gesichtsschutz tragen.* |
| P301+312 | IF SWALLOWED: Call a POISON CENTER/ doctor/…/ if you feel unwell.<br>*BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt/… anrufen.* |
| P302+352 | IF ON SKIN: Wash with plenty of water/…<br>*BEI BERÜHRUNG MIT DER HAUT: Mit viel Wasser/… waschen.* |
| P304+340 | IF INHALED: Remove person to fresh air and keep comfortable for breathing.<br>*BEI EINATMEN: Die Person an die frische Luft bringen und für ungehinderte Atmung sorgen.* |
| P305+351+338 | IF IN EYES: Rinse cautiously with water for several minuts. Remove contact lenses, if present and easy to do. Continue rinsing.<br>*BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser ausspülen. Eventuell vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter ausspülen.* |
| P330 | Rinse mouth.<br>*Mund ausspülen.* |
| P332+313 | If skin irritation occurs: Get medical advice/attention.<br>*Bei Hautreizung: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen.* |
| P333+313 | If skin irritation or rash occurs: Get medical advice/attention.<br>*Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen.* |

| | |
|---|---|
| P 337+313 | If eye irritation persists: Get medical advice/attention.<br>*Bei anhaltender Augenreizung: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen.* |
| P 342+311 | If experiencing respiratory symptoms: Call a POISON CENTER/doctor/…<br>*Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM/Arzt/… anrufen.* |
| P363 | Wash contaminated clothing before reuse.<br>*Kontaminierte Kleidung vor erneutem Tragen waschen.* |
| P370+378 | In case of fire: Use … to extinguish.<br>*Bei Brand: … zum Löschen verwenden.* |
| P403+235 | Store in a well-ventilated place. Keep cool.<br>*An einem gut belüfteten Ort aufbewahren. Kühl halten.* |

For further information please see Material Safety Data Sheets (***www.mn-net.com***).
*Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern (**www.mn-net.com**).*

The symbol shown on labels refers to further safety information in this section.
*Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.*

# 5 Protocols

## 5.1 RNA purification from cultured cells, laser captured cells, or microdissected cryosections

**Before starting the preparation:**

- Check if TCEP, Carrier RNA, rDNase, and Wash Buffer RA3 were prepared according to section 3.

**1 Supply sample**

Provide sample such as a pellet of up to $10^5$ cultured cells, laser captured cells or microdissected cryosections in a microcentrifuge tube (not provided).

For appropriate sample amounts see section 2.2.

**2 Lyse and homogenize cells**

Add **100 µL Buffer RA1** and **2 µL TCEP** to the cell sample and vortex vigorously (2 x 5 s).

If multiple samples are processed, the preparation of a master-premix is recommended (e.g., 1.1 mL Buffer RA1 and 22 µL TCEP for 10 preparations). Use 102 µL of the premix.

**+ 100 µL RA1**
**+ 2 µL TCEP**

This procedure is usually sufficient to homogenize cultured cells, laser captured cells, or microdissected cryosections. For further comments on homogenization methods see section 2.3.

**3 Add Carrier RNA**

Add **5 µL Carrier RNA working solution** (20 ng) to the lysate. Mix by vortexing (2 x 5 s). Spin down briefly (approx. 1 s 1000 x *g*) to clear the lid.

**+ 5 µL Carrier RNA**

**Mix**

*For preparation of Carrier RNA working solution see section 3*

**4 Filtrate lysate (*optional*)**

Place a **NucleoSpin® Filter** (violet ring) in a Collection Tube (2 mL; supplied), apply the mixture, and centrifuge for **30 s** at **11,000 x *g***.

**11,000 x *g***
**30 s**

*This step may be skipped when working with small amounts of sample, for example less than $10^5$ cells.*

**5 Adjust RNA binding condition**

Discard the **NucleoSpin® Filter** (violet ring). Add **100 µL ethanol (70 %)** to the homogenized lysate and mix by pipetting up and down (5 times).

**+ 100 µL 70 % EtOH**

**Mix**

*Alternatively, add 100 µL ethanol (70 %) to the sample in a 1.5 mL microcentrifuge tube (not provided) and mix by vortexing (2 x 5 s). Spin down briefly (approx. 1 s 1000 x g) to clear the lid. Pipette lysate up and down two times before loading the lysate.*

**6 Bind RNA**

For each preparation, take one **NucleoSpin® RNA XS Column** (light blue ring) placed in a Collection Tube. **Load** the lysate to the column. Centrifuge for **30 s** at **11,000 x *g***.

**Load lysate**

Place the column in a new Collection Tube (2 mL).

**11,000 x *g* 30 s**

*The maximum loading capacity of NucleoSpin® RNA XS Columns is 600 µL. Repeat the procedure if larger volumes are to be processed.*

**7 Desalt silica membrane**

Add **100 µL MDB** (Membrane Desalting Buffer) and centrifuge at **11,000 x *g*** for **30 s** to dry the membrane. It is not necessary to use a fresh Collection Tube after this centrifugation step.

**+ 100 µL MDB**

*Salt removal will make the following rDNase digest much more effective. If the column outlet has come into contact with the flow-through for any reason, discard the flow-through and centrifuge again for 30 s at 11,000 x g.*

**11,000 x *g* 30 s**

**8 Digest DNA**

Prepare **rDNase reaction mixture** in a sterile microcentrifuge tube (not provided): for each isolation, add **3 µL** reconstituted rDNase (also see section 3) to **27 µL Reaction Buffer for rDNase**. Mix by flicking the tube.

**+ 25 µL rDNase reaction mixture**

Apply **25 µL rDNase reaction mixture** directly onto the center of the silica membrane of the column. Close the lid. Incubate at **room temperature** for **15 min**.

**RT 15 min**

*It is not necessary to use a new Collection Tube after the incubation step.*

**9 Wash and dry silica membrane**

**1st wash**

Add **100 µL Buffer RA2** to the NucleoSpin® RNA XS Column. Incubate for **2 min** at **RT**. Centrifuge for **30 s** at **11,000 x *g***.

**+ 100 µL RA2**

**RT 2 min**

Place the column into a new Collection Tube (2 mL).

*Buffer RA2 will inactivate the rDNase.*

**11,000 x *g* 30 s**

**2nd wash**

Add **400 µL Buffer RA3** to the NucleoSpin® RNA XS Column. Centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and place the column back into the Collection Tube.

**+ 400 µL RA3**

*Note: Make sure that residual buffer from the previous steps is washed away with Buffer RA3, especially if the lysate has been in contact with the inner rim of the column during loading of the lysate onto the column. For efficient washing of the inner rim flush it with Buffer RA3.*

**11,000 x *g* 30 s**

**3rd wash**

Add **200 µL Buffer RA3** to the NucleoSpin® RNA XS Column. Centrifuge for **2 min** at **11,000 x *g*** to dry the membrane. Place the column into a nuclease-free Collection Tube (1.5 mL; supplied).

**+ 200 µL RA3**

**11,000 x *g* 2 min**

*If for any reason the liquid level in the Collection Tube has reached the NucleoSpin® RNA XS Column after centrifugation, discard flow-through and centrifuge again.*

**10 Elute highly pure RNA**

Elute the RNA in **10 µL $H_2O$** (RNase-free; supplied) and centrifuge at **11,000 x *g*** for **30 s**.

**+ 10 µL RNase-free $H_2O$**

*If higher RNA concentrations or higher elution volumes are desired, elution volume may be varied in the range of 5–30 µL.*

**11,000 x *g* 30 s**

*For further details on alternative elution procedures see section 2.4.*

## 5.2 RNA purification from tissue

**Before starting the preparation:**

- Check if TCEP, Carrier RNA, rDNase, and Wash Buffer RA3 were prepared according to section 3.

**1 Supply sample**

Provide tissue sample such as a biopsy in a microcentrifuge tube (not provided).

For appropriate sample amounts see section 2.2.

**2 Lyse and homogenize tissue**

Add **200 µL Buffer RA1** and **4 µL TCEP** to the tissue sample and vortex vigorously (2 x 5 s).

**+ 200 µL RA1**
**+ 4 µL TCEP**

Disruption with a rotor-stator homogenizer or with a shaker and steel balls are recommended methods for the homogenization of tissue samples. For further comments on homogenization methods see section 2.3.

If multiple samples are processed, the preparation of a master-premix is recommended (e.g., 2.2 mL Buffer RA1 and 44 µL TCEP for 10 preparations). Use 204 µL of the premix.

**3 Add Carrier RNA**

Add **5 µL Carrier RNA working solution** (20 ng) to the lysate. Mix by vortexing (2 x 5 s). Spin down briefly (approx. 1 s 1000 x *g*) to clear the lid.

**+ 5 µL Carrier RNA**

**Mix**

*For preparation of Carrier RNA working solution see section 3.*

**4 Filtrate lysate**

Reduce viscosity and clear the lysate by filtration through **NucleoSpin® Filter** (violet ring): Place the NucleoSpin® Filter (violet ring) in a Collection Tube (2 mL; provided), apply the mixture, and centrifuge for **30 s** at **11,000 x *g***.

**11,000 x *g***
**30 s**

In case of visible pellet formation (depending on sample amount and nature), transfer supernatant without any formed pellet to a new 1.5 mL microcentrifuge tube (not included).

**5 Adjust RNA binding condition**

Discard the NucleoSpin® Filter (violet ring), add **200 µL ethanol (70 %)** to the homogenized lysate and mix by pipetting up and down (5 times).

Alternatively, transfer flow-through into a new 1.5 mL microcentrifuge tube (not provided), add **200 µL ethanol** (70 %), and mix by vortexing (2 x 5 s). Spin down briefly (approx. 1 s 1000 x *g*) to clear the lid. Pipette lysate up and down two times before loading the lysate.

**+ 200 µL 70 % EtOH**

**Mix**

*After addition of ethanol a stringy precipitate may become visible which will not affect the RNA isolation. Be sure to disaggregate any precipitate by mixing and load all of the precipitate on the column as described in step 6. Do not centrifuge the ethanolic lysate before loading it onto the column in order to avoid pelleting the precipitate.*

**6 Bind RNA**

For each preparation, take one **NucleoSpin® RNA XS Column** (light blue ring) placed in a Collection Tube and load the lysate to the column. Centrifuge for **30 s** at **11,000 x *g***. Place the column in a new Collection Tube (2 mL).

**Load lysate**

**11,000 x *g* 30 s**

*The maximum loading capacity of NucleoSpin® RNA XS Columns is 600 µL. Repeat the procedure if larger volumes are to be processed.*

**7 Desalt silica membrane**

Add **100 µL MDB** (Membrane Desalting Buffer) and centrifuge at **11,000 x *g*** for **30 s** to dry the membrane. It is not necessary to use a fresh Collection Tube after this centrifugation step.

**+ 100 µL MDB**

**11,000 x *g* 30 s**

*Salt removal will make the following rDNase digest much more effective. If the column outlet has come into contact with the flow-through for any reason, discard the flow-through and centrifuge again for 30 s at 11,000 x g.*

**8 Digest DNA**

Prepare **rDNase reaction mixture** in a sterile microcentrifuge tube (not provided): for each isolation, add **3 µL** reconstituted rDNase (also see section 3) to **27 µL Reaction Buffer for rDNase**. Mix by flicking the tube.

**+ 25 µL rDNase reaction mixture**

Apply **25 µL rDNase reaction mixture** directly onto the center of the silica membrane of the column. Close the lid. Incubate at **room temperature** for **15 min**.

**RT 15 min**

*It is not necessary to use a new Collection Tube after the incubation step.*

**9 Wash and dry silica membrane**

**1st wash**

**+ 100 µL RA2**

Add **100 µL Buffer RA2** to the NucleoSpin® RNA XS Column. Incubate for **2 min** at **RT**. Centrifuge for **30 s** at **11,000 x *g***.

**RT 2 min**

**11,000 x *g* 30 s**

Place the column into a new Collection Tube (2 mL).

*Buffer RA2 will inactivate the rDNase.*

**2nd wash**

**+ 400 µL RA3**

Add **400 µL Buffer RA3** to the NucleoSpin® RNA XS Column. Centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and place the column back into the Collection Tube.

**11,000 x *g* 30 s**

**3rd wash**

**+ 200 µL RA3**

Add **200 µL Buffer RA3** to the NucleoSpin® RNA XS Column. Centrifuge for **2 min** at **11,000 x *g*** to dry the membrane. Place the column into a nuclease-free Collection Tube (1.5 mL; supplied).

**11,000 x *g* 2 min**

*If for any reason the liquid level in the Collection Tube has reached the NucleoSpin® RNA XS Column after centrifugation, discard flow-through and centrifuge again.*

**10 Elute highly pure RNA**

Elute the RNA in **10 µL $H_2O$** (RNase-free; supplied) and centrifuge at **11,000 x *g*** for **30 s**.

*If higher RNA concentrations or higher elution volumes are desired, elution volume may be varied in the range of 5–30 µL.*

*For further details on alternative elution procedures see section 2.4.*

**+ 10 µL RNase-free H2O**

**11,000 x *g* 30 s**

## 5.3 Clean-up and concentration of RNA

**Before starting the preparation:**

- Check if Wash Buffer RA3 were prepared according to section 3.

**1 Supply sample**

Provide **up to 300 µL sample** such as prepurified RNA (e.g., phenol purified) or RNA from reaction mixtures (e.g., labelling reactions) in a microcentrifuge tube (not provided).

For appropriate sample amounts see section 2.2.

**Sample**

**2 Prepare lysis-binding buffer premix**

For every 100 µL of sample combine **25 µL Buffer RA1** with **75 µL ethanol (96–100 %)** and mix.

If processing multiple samples, the preparation of a master-premix (1 volume Buffer RA1 plus 3 volumes ethanol 96–100 %) is recommended.

**+ 25 µL RA1**
**+ 75 µL EtOH (96–100 %) per 100 µL sample**

**Mix**

**3 Add Carrier RNA**

Not necessary!

**4 Filtrate lysate**

Not necessary!

**5 Adjust RNA binding condition**

Add **one volume of premix** to the sample (e.g., 100 µL premix to a 100 µL sample) and mix (2 x 5 s). If necessary, spin down briefly (approx. 1 s 1000 x *g*) to clear the lid.

**Add 1 vol. premix to sample**

**Mix**

**6 Bind RNA**

For each preparation, take one **NucleoSpin® RNA XS Column** (light blue ring) placed in a Collection Tube and **load the lysate** to the column. Centrifuge for **30 s** at **11,000 x *g***.

For samples > 300 µL, load in two steps.

Place the column in a new Collection Tube (2 mL).

*For high demanding applications, the recovery rate can be increased as follows: Centrifuge 30 s at 2,000 x g prior to centrifugation for 30 s at 11,000 x g.*

**Load lysate**

**11,000 x *g***
**30 s**

**7 Desalt silica membrane**

Not necessary!

**8 Digest DNA**

Not necessary!

**9 Wash and dry silica membrane**

**1st wash**

Add **400 µL Buffer RA3** to the NucleoSpin® RNA XS Column. Centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and place the column back into the Collection Tube.

**+ 400 µL RA3**

**11,000 x *g***
**30 s**

**2nd wash**

Add **200 µL Buffer RA3** to the NucleoSpin® RNA XS Column. Centrifuge for **2 min** at **11,000 x *g*** to dry the membrane. Place the column into a nuclease-free Collection Tube (1.5 mL; supplied).

**+ 200 µL RA3**

**11,000 x *g***
**2 min**

*If for any reason the liquid level in the Collection Tube has reached the NucleoSpin® RNA XS Column after centrifugation, discard flow-through and centrifuge again.*

**10 Elute highly pure RNA**

Elute the RNA in **10 µL $H_2O$** (RNase-free; supplied) and centrifuge at **11,000 x g** for **30 s**.

**+ 10 µL RNase-free $H_2O$**

**11,000 x *g***
**30 s**

*If higher RNA concentrations or higher elution volumes are desired, elution volume may be varied in the range of 5–30 µL.*

*For further details on alternative elution procedures see section 2.4.*

## 5.4 rDNase digestion in the eluate

The on-column rDNase digestion in the standard protocol is very efficient and thus results in minimal residual DNA. This DNA will not be detectable in most downstream applications. However, removal of DNA to a completely undetectable level is challenging and the efficiency of an on-column DNA digestion is sometimes not sufficient for downstream applications requiring lowest residual content of DNA.

A typical example for such a demanding application is an RT-PCR reaction in which the primer molecules do not differentiate between cDNA (derived from RNA) and contaminating genomic DNA. Especially, if

- high copy number targets are analyzed (e.g., multi gene family, mitochondrial, plastidal or plasmid targets (from transfections))
- the target gene is of a very low expression level
- the amplicon is relatively small (< 200 bp).

DNA digestion in solution can efficiently destroy contaminating DNA. However, stringent RNase control and subsequent repurification of the RNA (in order to remove buffer, salts, DNase and digested DNA) are usually required.

The high quality, recombinant, RNase-free DNase (rDNase) in the **NucleoSpin® RNA XS** kits facilitates such a digestion in solution in order to remove even traces of contaminating DNA.

| | |
|---|---|
| **A** | **Digest DNA (Reaction setup)**<br><br>Prepare enzyme-buffer premix: Add **1 µL rDNase** to **10 µL Reaction Buffer for rDNase.**<br><br>Add 1/10 volume of enzyme-buffer premix to the eluted RNA (e.g., to 10 µL RNA add 1 µL of the premix comprising buffer and enzyme). |
| **B** | **Incubate sample**<br><br>Incubate for **10 min** at **37 °C**. |

**C** **Repurify RNA**

Repurify RNA with a suitable RNA cleanup procedure, for example following section 5.3, by ethanol precipitation or with the NucleoSpin® RNA Clean-up XS kit (see ordering information).

**Ethanol precipitation, exemplary**

Add 0.1 volume of 3 M sodium acetate, pH 5.2 and 2.5 volumes of 96–100 % ethanol to one volume of sample. Mix thoroughly.

Incubate several minutes to several hours at -20 °C or 4 °C, respectively.

*Note: Choose long incubation times if the sample contains low RNA concentration. Short incubation times are sufficient if the sample contains high RNA concentration.*

Centrifuge for 10 min at max. speed.

Wash RNA pellet with 70 % ethanol.

Dry RNA pellet and resuspend RNA in RNase-free $H_2O$.

# 6 Appendix

## 6.1 Troubleshooting

| Problem | Possible cause and suggestions |
|---|---|
| RNA is degraded/ no RNA obtained | *RNase contamination*<br>• Create an RNase free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes is recommended. Keep tubes closed whenever possible during the preparation. Glassware should be oven-baked for at least 2 hours at 250 °C before use. |
| Poor RNA quality or yield | *Reagents not applied or restored properly*<br>• Reagents not properly restored. Add the indicated volume of RNase-free $H_2O$ to rDNase vial and 96 % ethanol to Buffer RA3 Concentrate and mix. Reconstitute and store lyophilized rDNase according to instructions given in section 3.<br>• Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added.<br>• No ethanol has been added after lysis. Binding of RNA to the silica membrane is only effective in the presence of ethanol.<br>*Kit storage*<br>• Reconstitute and store lyophilized rDNase according to instructions given in section 3.<br>• Store other kit components at room temperature. Storage at low temperatures may cause salt precipitation.<br>• Keep bottles tightly closed in order to prevent evaporation or contamination.<br>*Ionic strength and pH influence $A_{260}$ absorption as well as ratio $A_{260}/A_{280}$*<br>• For adsorption measurement, use 5 mM Tris pH 8.5 as diluent. Please see also:<br>- Manchester, K L. 1995. Value of $A_{260}/A_{280}$ ratios for measurement of purity of nucleic acids. Biotechniques 19, 208 - 209.<br>- Wilfinger, W W, Mackey, K and Chomczyski, P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques 22, 474 - 481. |

| Problem | Possible cause and suggestions |
|---|---|
| Poor RNA quality or yield *(continued)* | *Sample material*<br>• Sample material not stored properly. Whenever possible, use fresh material. If this is not possible, flash freeze the samples in liquid $N_2$. Samples should always be kept at -70 °C. Never allow tissues to thaw before addition of Buffer RA1. Perform disruption of samples immediately after addition of Lysis Buffer RA1.<br>• Insufficient disruption and / or homogenization of starting material. Ensure thorough sample disruption and use NucleoSpin® Filters for easy homogenization of disrupted starting material. |
| Low $A_{260}/A_{230}$ ratio | *Carry-over of guanidinium thiocyanate*<br>• Carefully load the lysate to the NucleoSpin® RNA II Column and try to avoid a contamination of the upper part of the column and the column lid.<br>• Make sure that residual Wash Buffer RA2 is washed away with Wash Buffer RA3. This may be done by applying Buffer RA3 to the inner rim of the column. |
| Clogged NucleoSpin® Column/ Poor RNA quality or yield | *Sample material*<br>• Too much starting material used. Overloading may lead to decreased overall yield. Reduce amount of sample material or use larger volume of Buffer RA1.<br>• Insufficient disruption and/or homogenization of starting material. Ensure thorough sample disruption and use NucleoSpin® Filters for easy homogenization of disrupted starting material. |
| Contamination of RNA with genomic DNA | *rDNase not active*<br>• Reconstitute and store lyophilized rDNase according to instructions given in section 3.<br>*DNase solution not properly applied*<br>• Pipette rDNase solution directly onto the center of the silica membrane and close the lid.<br>*Too much cell material used*<br>• Reduce quantity of cells or tissue used. |

| Problem | Possible cause and suggestions |
|---|---|
| Contamination of RNA with genomic DNA *(continued)* | *DNA detection system too sensitive*<br>• The amount of DNA contamination is effectively reduced during the on-column digestion with rDNase. Anyhow, it can not be guaranteed that the purified RNA is 100 % free of DNA, therefore in very sensitive applications it might still be possible to detect DNA.<br>The probability of DNA detection with PCR increases with:<br>- the number of DNA copies per preparation: single copy target < plastidial/mitochondrial target < plasmid transfected into cells<br>- decreasing of PCR amplicon size.<br>• Use larger PCR targets (e.g., > 500 bp) or intron spanning primers if possible.<br>• **Use support protocol 5.4 for subsequent rDNase digestion in solution.** |
| Suboptimal performance of RNA in downstream experiments | *Carry-over of ethanol or salt*<br>• Do not let the flow-through touch the column outlet after the second Buffer RA3 wash. Be sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic Buffer RA3 completely.<br>• Check if Buffer RA3 has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by Buffer RA3.<br>• Depending on the robustness of the used RT-PCR system, RT-PCR might be inhibited if complete eluates are used as template for RT-PCR. Use less eluate as template.<br>*Store isolated RNA properly*<br>• Eluted RNA should always be kept on ice for optimal stability since trace contaminations of omnipresent RNases (general lab ware, fingerprints, dust) will degrade the isolated RNA. For short term storage freeze at -20 °C, for long term storage freeze at -70 °C. |

| Problem | Possible cause and suggestions |
|---|---|
| Discrepancy between $A_{260}$ quantification values and PCR quantification values | *Silica abrasion from the membrane*<br>• Due to the typically low RNA content in very small samples and the resulting low total amount of isolated RNA, an RNA quantification via $A_{260}$ absorption measurement is often hampered due to the low sensitivity of the absorption measurement. When performing absorption measurements close to the detection limit of the photometer, the measurement may be influenced by minor amounts of silica abrasion. In order to prevent incorrect $A_{260}$-quantification of small RNA amounts centrifuge the eluate for 30 s at > 11.000 x *g* and take an aliquot for measurement without disturbing any sediment. Alternatively, use a silica abrasion insensitive RNA quantification method (e.g., RiboGreen fluorescent dye). |
| Unexpected $A_{260}/A_{280}$ ratio | *Measurement not in the range of photometer detection limit*<br>• In order to obtain a significant $A_{260}/A_{280}$ ratio it is necessary that the initially measured $A_{260}$ and $A_{280}$ values are significantly above the detection limit of the photometer used. An $A_{280}$ value close to the background noise of the photometer will cause unexpected $A_{260}/A_{280}$ ratios. |

## 6.2 Ordering information

| Product | REF | Pack of |
|---|---|---|
| NucleoSpin® RNA XS | 740902.10/.50/.250 | 10/50/250 |
| NucleoSpin® RNA Clean-up XS | 740903.10/.50/.250 | 10/50/250 |
| NucleoSpin® totalRNA FFPE XS | 740969.10/.50/.250 | 10/50/250 |
| NucleoSpin® RNA | 740955.10/.50/.250 | 10/50/250 |
| NucleoZOL | 740404.200 | 200 mL |
| NucleoSpin® RNA Blood | 740200.10/.50 | 10/50 |
| NucleoSpin® totalRNA FFPE | 740982.10/.50/.250 | 10/50/250 |
| NucleoSpin® RNA Midi | 740962.20 | 20 |
| NucleoSpin® RNA/Protein | 740933.10/.50/.250 | 10/50/250 |
| NucleoSpin® TriPrep | 740966.10/.50/.250 | 10/50/250 |
| NucleoSpin® RNA Clean-up | 740948.10/.50/.250 | 10/50/250 |
| NucleoSpin® RNA/DNA Buffer Set | 740944 | Suitable for 100 preps |
| Buffer RA1 | 740961<br>740961.500 | 60 mL<br>500 mL |
| rDNase Set | 740963 | 1 set |
| Reducing Agent TCEP | 740395.107 | 107 mg |
| NucleoSpin® Filters | 740606 | 50 |
| Collection Tubes (2 mL) | 740600 | 1000 |

Visit ***www.mn-net.com*** for more detailed product information.

## 6.3 References

**Fleige** S, Pfaffl MW.: RNA integrity and the effect on the real-time qRT-PCR performance. Mol Aspects Med. 2006 Apr-Jun; 27(2-3):126-39. Epub 2006 Feb 15. Review.

**Imbeaud** S, Graudens E, Boulanger V, Barlet X, Zaborski P, Eveno E, Mueller O, Schroeder A, Auffray C.: Towards standardization of RNA quality assessment using user-independent classifiers of microcapillary electrophoresis traces. Nucleic Acids Res. 2005 Mar 30;33(6):e56.

**Miller** CL, Diglisic S, Leister F, Webster M, Yolken RH.: Evaluating RNA status for RT-PCR in extracts of postmortem human brain tissue. Biotechniques. 2004 Apr; 36(4):628-33.

**Schoor** O, Weinschenk T, Hennenlotter J, Corvin S, Stenzl A, Rammensee HG, Stevanovic S.: Moderate degradation does not preclude microarray analysis of small amounts of RNA. Biotechniques. 2003 Dec; 35(6):1192-6, 1198-201.

## 6.4 Product use restriction/warranty

**NucleoSpin® RNA XS** kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for IN-VITRO-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for IN-VITRO-diagnostic use. Please pay attention to the package of the product. IN-VITRO-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR IN-VITRO-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer´s sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or components not manufactured by MACHEREY-NAGEL, or damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

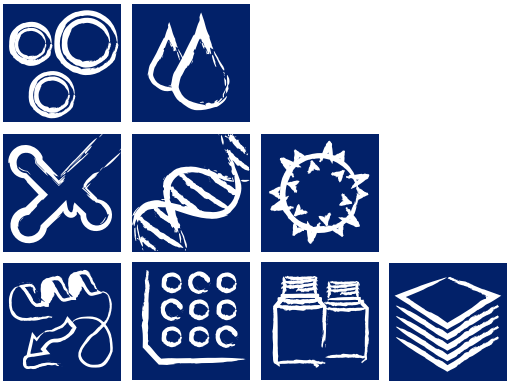
The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL´s sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL´s employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You

may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Last updated: 07/2010, Rev. 03

Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 24 21 969-270
tech-bio@mn-net.com



MACHEREY-NAGEL

**MACHEREY-NAGEL GmbH & Co. KG** · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany

**Germany and international:**
Tel.: +49 24 21 969-0
E-mail: info@mn-net.com

**Switzerland:**
**MACHEREY-NAGEL AG**
Tel.: +41 62 388 55 00
E-mail: sales-ch@mn-net.com

**France:**
**MACHEREY-NAGEL EURL**
Tel.: +33 388 68 22 68
E-mail: sales-fr@mn-net.com

**USA:**
**MACHEREY-NAGEL Inc.**
Tel.: +1 484 821 0984
E-mail: sales-us@mn-net.com

A034939/0951.5